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Breast cancer is one of the leading causes of cancer deaths of women in the United States. Fortunately, this disease is no longer a "black box" that can only be studied empirically. Recent advances in understanding of normal mammary development and carcinogenic processes have identified a number of specific genes and processes that are dysregulated in breast cancer. This means that research on breast cancer has finally advanced to the stage where a concentrated effort in translational research will yield great strides in detection, diagnosis, and treatment. The Molecular Medicine graduate training program at Yale was recently developed to address these issues. This program was developed to offer an interdisciplinary course of study that will foster an integrated view of disease, built upon a rigorous foundation of basic sciences. The emphasis on disease mechanisms and translational research is unique to Molecular Medicine, and distinguishes it from other pre-doctoral programs at Yale. The Predoctoral Training Program in Breast Cancer Research will recruit individuals interested in careers in breast cancer research to the Molecular Medicine Program, provide specialist training in breast cancer-specific areas, and integrate their training experience with basic scientists and clinicians investigating

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INTRODUCTION

The past decade has witnessed a revolution in the power of biologists to investigate fundamental mechanisms underlying disease. This change has resulted from major advances in biological research, coupled with the extraordinary power of modern molecular genetics for identification of gene mutations in disease. The result is that investigation of many human diseases has gone beyond the descriptive level to the root causes. This new knowledge means that the tools of genetics, immunology, cell biology, molecular biology, and other disciplines can now be combined to investigate disease pathogenesis, and to apply these findings to issues of diagnosis and treatment. The Molecular Medicine graduate training program at Yale was recently developed to address these issues. This program was developed to offer an interdisciplinary course of study that will foster an integrated view of disease, built upon a rigorous foundation of basic sciences. The emphasis on disease mechanisms and translational research is unique to Molecular Medicine, and distinguishes it from other pre-doctoral programs at Yale. The Predoctoral Training Program in Breast Cancer Research will recruit individuals interested in careers in breast cancer research to the Molecular Medicine Program, provide specialist training in breast cancer-specific areas, and integrate their training experience with basic scientists and clinicians investigating breast cancer at Yale.

BODY

We are now completing the second year of this new training program.

Recruitment. Class entering Fall 2000. As discussed in the proposal, trainees are recruited through the Pharmacological Sciences and Molecular Medicine Program of the university-wide combined BBS training program. In last year's progress report we provided a copy of the poster that was used as advertising for this program (distributed to 3500 academic units), and a link to our web site advertising this program. Students chosen for funding by the program in the first year had been those whom we felt were most likely to remain in cancer research from the entering class. Since the training program was in place during recruitment for the second year of students, we attempted to have students self-identify as appropriate for this training program. Although we were successful in identifying individuals who opted to specialize in cancer research, it was difficult to ensure that there was a narrow disease-specific focus on breast cancer.

Training. The first year in graduate school consists of course work and a series of research rotations. All incoming students were oriented along with other students in the Pharmacological Sciences and Molecular Medicine Track, and assigned to trainers and Track Directors William Sessa and David Stern as advisors. The advisors met with the students to jointly plan out a curriculum for the first year. In year 1, this consists of course work and three laboratory rotations. The rotations serve to train individuals in design, execution, and interpretation of laboratory research projects, to expose the trainees to a variety of research experiences, and to enable trainees to identify compatible dissertation advisors. Students are required to choose a dissertation advisor the end of the first year.

Course work. As discussed in the proposal, the purpose of coursework is to ensure that students have a strong basic science foundation that complements undergraduate coursework, and to provide students with advanced and specialty knowledge. This process continued in year 2, with inclusion of breast-cancer-specific training. Classes taken by the first year students are described in Table 3, and include "foundation" courses such as cell biology and genetics, and more specialized coursework in cancer and disease mechanisms.

As a component of the Breast Cancer Research Training Program, we offered a class, Spring, 2001, "Biology and Therapy of Breast Cancer" (Fig. 1). The class was organized by trainers DiGiovanna, Stern, and Languino. It was open to undergraduates, graduate students, and interested fellows, with the majority of students being first and second year Yale Ph.D. students. The overall goal of the class was to introduce students to important problems in breast cancer research, and to attempt to integrate the basic biology of the mammary gland with the problem of breast cancer. Since PhD students have little exposure to clinical issues, we also endeavored to provide direct

exposure to clinical aspects of breast cancer. This is unusual and innovative. The approach included attendance at the Yale Comprehensive Cancer Center Breast Cancer Research Program Conferences (BCRP on the schedule in Fig. 1), laboratory exposure to breast cancer gross and histological specimens, and observing the breast cancer clinical conference tumor board, where clinical decisions were made.

Updated 3/23/01 **Biology and Therapy of Breast Cancer**

Path 660b Spring 2001

Meeting times	**Mondays & Thursdays,**		**1:30pm - 2:30pm**
Meeting place	**Brady Memorial Lab, 310 Cedar Street**		**rm. BML-137**
Organizers	**DiGiovanna, M.P. NSB-288 737-5240**		
	Languino, L.R. BML-165 73-1454		
	Stern, D.F.	**BML-342a**	**785-4832**
Administration	**Falato, JoAnn**	**BML-342**	**785-6721**

1	January 8	M	DiGiovanna	Clinical Intro to Breast Cancer
2	January 9	T		**BCRP***
3	January 15	M	D Rimm (or D.Dillon)	Pathology of Breast Cancer
4	January 18	R	F. Naftolin	Endocrine regulation of the mammary gland
5	January 22	M	D Rimm	lab for pathology
6	January 25	R	Languino	Mammary development, including Bissell work
7	January 29	M	Languino	readings
	February 1	R	**Stern**	Oncogenes/TS genes I cytogenetics, growth regulators
	February 5	M	Stern	readings
	February 8	R	Stern	Oncogenes/TS genes II DNA damage/p53
	February 12	M	Stern	readings
	February 15	T	Languino	readings on Stromal/epithelial interactions
	February 19	M	Matloff/DiG	Brca1/Brca2 genetics, clinical challenges, testing and patient management issues
	February 22	R	DiGiovanna	readings-breast Ca prevention trial; mastectomy trial
	February 26	M	Perkins	Rodent Models for Breast Cancer
	March 1	R	Languino	readings application of mouse models
	March 19	M	**Madri**	Angiogenesis
	March 22	R	Madri	readings
	March 26	M	Languino	**Invasion/metastasis**
	March 29	**R**	**Languino**	readings
	April 2	M	**Altieri**	**Apoptosis**
	April 5	R	**Altieri**	readings
	April 9			
	April 10	T	Weinberg seminar	
	April 16	M	DiGiovanna	therapeutics, present, theory, application, and problems
	April 19	R	Fu	Stats
	April 23	M	Undergraduate Students	FINAL
	****April 25	W	DiGiovanna	5:00 PM Clinical conference tumor board****
	April 30	M	Rimm	handling diagnostic challenges: present and future
	May 3	R	Crews	therapeutics
	May 7	M	**Crews**	**readings**
	May 8	T		**BCRP**

***BCRP** Tuesdays at 5:30 with dinner

Fig. 1. Syllabus for the Breast Cancer Class.

Year 1, rotations. As members of the BBS, students are free to rotate in any of the participating BBS labs at the university, which includes most labs engaged in biomedical research. The rotation advisors and rotation topics for students who were entered during this period (2000-2001) are shown in Table 2. Six of the 14 rotations were done in labs of trainers (indicated by double asterisk **).

A description of rotation projects follows.

Seda Eminaga

David Stern Domain analysis of DNA damage checkpoint gene Rad53
David Rimm Alpha-catenin interactions with E-cadherin
Anton Bennett Detection of activated forms of small GTPases

Leigh-Ann Higa

Archibald Perkins cDNA array analysis of transcriptional targets of myeloid oncogene *EVI1*
Anglique Bordey Electrophysiology of lactate transporter in astrocytes
Mark Shlomchik ELISA spot assay for autoimmune antibodies
Hui Zhang Involvement of double-strand break repair complex in DNA damage response

Kristen Massimine

Elias Lolis Identification of an expression system for vMIP-II
Richard Lifton Elucidation of an antagonists for a mutant mineralcorticoid receptor
Daniel DiMaio Senescence in cervical carcinoma cells

Alexander Urban

Stephen Strittmatter Microarray analysis of axotomy-induced changes in gene expression
Paul Lizardi Linear amplification of mRNA for microarry analysis by SMART method
Pasko Rakic Effects of hypoxia on mitosis in developing mouse brain

First year students: selection of dissertation advisers. Since, under the BBS program, students are free to rotate throughout the university, it was inevitable that there would be some attrition between years one and two. Of the four first-year students funded in year two of the program, two (KM and AU) affiliated with faculty outside of the program. The other two, Leigh-Ann Higa and Seda Eminaga have remained with the training faculty.

Trainees and their projects.

Students in year 2, 2000-2001.

Soo-Jung Lee. Ms. Lee continues her work in the laboratory of the program director, Dr. Stern. The major components of DNA checkpoint pathways are conserved between budding yeast and humans. The Stern lab is investigating DNA checkpoint pathways connecting DNA damage to activation of the yeast ortholog of human Atm (yeast Mec1), which in turn activates human Chk2 (yeast Rad53). Ms. Lee is presently working on in vitro activation of Rad53 activation. by the Atm homolog Mec1. Thus, studying the mechanism of RAD53 activation by Mec1/Tel1-Rad9 in yeast will provide important clues to understanding well conserved DNA damage checkpoint signaling pathway.

Breast cancer relevance. This work is directly relevant to breast cancer, since it is now clear that Chk2 is an intermediary linking DNA checkpoint pathways from candidate breast cancer tumor suppressor Atm to breast cancer tumor suppressor p53; since Chk2 phosphorylates and modulates Brca1 function, and since Chk2 mutations are found in variant p53+ forms of Li-Fraumeni syndrome, which predisposes to breast cancer and other cancers.

Julie Wu. Ms. Wu is working in the laboratory of trainer Anton Bennett. Mitogen activated protein kinase (MAP) phosphatase 1 (MKP1) is a dual specificity tyrosine/threonine phosphatase that is highly regulated by growth regulators and stress. It dephosphorylates MAPK family members. MKP1 regulates the activities of p38 (Stress activated protein kinase) and ERKs. Attenuation of MAPK activity after activation is a tightly regulated process.

MKP-1 is protein dual specific protein phosphatases that regulates cellular proliferation and apoptosis through dephosphorylation and inactivation the MAPK family members, ERK, JNK and p38. MKP-1 is an immediate early gene that is regulated by growth factors and stress. It has been shown that MKP-1 expression is increased in hepatocytes during liver regeneration and osmotic stress. The role of MKP-1 in hepatocytes has not been examined. Ms. Wu's project has two components. (1) She isdetermining how EGF immobilization of cytoplasmic and nuclear calcium regulates MKP-1 gene expression and ERK activation. In hepatocytes, activation of the EGFR increases calcium levels in the cytoplasm as well as in the nucleus. Overexpression of a calcium chelating protein parvalbumin fused to a nuclear exclusion or nuclear localization sequence is used to inhibit EGF mobilization of calcium in the cytoplasm or nucleus respectively. ERK activity and the transactivation activity of its substrate, Elk-1 in response to EGF stimulation are measured. MKP-1 promoter activity is measured as a read out for MKP-1 expression. (2) The role of MKP-1 in vivo has not been examined. MKP-1 knockout mice have been generated, and no apparent phenotype was observed. Over the last year, She has been increasing the size of the MKP-1 knockout mice colony. Preliminary biochemical analysis of primary hepatocytes isolated from wild type or MKP-1 knockout mice has been performed.
Breast cancer relevance. MAPKs are major regulators of cell growth and apoptosis. Oncogenes such as Ras and ErbB2 function in part through activation of MAPKs. Regulation of MAPK attenuation may be as important as regulation of activation. Inability to attenuate the activity of activated proteins results in abnormal biological processes. Inability to attenuate a p38 MAPK activity during stress may uncouple the cell's ability to respond to stress and repair induced damage. This may result in gene mutations, and/or unregulated growth, two hallmarks of cancer.

Jessica Hawes. Ms. Hawes began this year working on a stem cell project. She has now begun to investigate FRS2-dependent and independent fibroblast growth factor signaling. The goal is to determine functional differences between FRS2 and the related FRS1. She will do this using NGF-FGFR1 chimeras containing the extracellular domain of NGF and the transmembrane and intracellular domains of FGFR1. She is also preparing mutant NGF-FGFR1 chimeras which are unable to bind FRS2. She will then examine the differences and similarities of the wild-type and mutant chimeras in wild-type and FRS2 -/- fibroblasts.

Breast cancer relevance. Fibroblast growth factors are important growth regulators in normal and malignant mammary tissue. FRS2 is a major mediator of FGF responses, and is relatively specific to this receptor system. Learning about the FRS2-regulated pathways will help clarify the mechanisms of FGF-regulated mammary proliferation, and may lead to therapeutics that target this pathways.

Students in year 1, 2000-2001 who have remained in the training program. (beginning dissertation projects)

Seda Eminaga. Ms. Eminaga is identifying substrates for protein tyrosine phosphatase SHP-2. SHP2 is a widely expressed protein that has been shown to be a positive transducer of signaling pathways such as MAPK, PDGF, EGF, and Insulin pathways. In addition, there are reports that suggest that SHP-2 is involved in cell migration, adhesion and angiogenesis. SHP-2 has also

been shown to be downstream of IL-6 which is known to support cell growth and prevent apoptosis.
However, the physiological substrates for SHP-2 are not known. My project will be to identify potential substrates for SHP-2 and therefore, understand better how SHP-2 is involved in the regulation of above pathways.
Breast cancer relevance. SHP2 is activated by many growth activating receptor kinases, including EGFR, IGF1R, and ErbB2. These studies will clarify how SHP-2 functions in growth regulation. Other processes regulated by SHP-2, migration, adhesion, and angiogenesis, are important in invasion and metastasis. Understanding how these pathways work have important therapeutic implications in cancer and, as a positive growth regulator, SHP-2 is a potential target for drug development.

Leigh-Ann Higa. Ms. Higa is investigating the link between DNA damage, repair, and replication. In particular, She is focusing on the activity of the Nbs1-Mre11-Rad50 protein complex following irradiation of mammalian cultured cells. This complex is a central regulator of the DNA damage response, and alteration of its form or function may contribute to genomic instability and oncogenesis.
Breast cancer relevance. This protein complex is an important intermediary in DNA checkpoint regulation, with downstream components including p53. NBS1 is itself a tumor suppressor gene, that when mutated leads to the carcinogenic Nijmegan Breakage Syndrome. Understanding this pathway is essential for understanding genome destabilization, an important component of carcinogenesis. It may also aid in better using existing genotoxic anti-cancer drugs, since these genes are an important component of the protective response to DNA damage.

In year 2 these students will complete coursework, including breast cancer training, qualifying exams, and will begin to lay the foundation for the dissertation. Although the students have dissertation advisors, the dissertation project is not formalized until the prospectus exam, which must be completed by the end of year 3. A brief description of research directions of the five students is now provided, with the caveat that it may be some time before the projects fully evolve.

Accomplishments

- Recruitment of a highly qualified group of students interested in cancer research.
- Guiding students through an appropriate series of classes, including training in cell biology, disease mechanisms, cancer, and pharmacology.
- Creating a new class "Biology and Therapy of Breast Cancer" for trainees and other members of the Yale community.
- Providing students with research rotations relevant to cancer research.
- Selection of second year students with dissertation projects relevant to breast cancer research for further training through the program.

Reportable outcomes.

None.

Last	First	Institution	Degree(s)	GPA	Verbal	Quant	Analytical
Anyatonwu	Georgia	City College of New York (Cun	BS	3.46	350	550	540
Basavapathruni	Aravind	Illinois, Univ Of--Champ/Urbn	BS	3.65	490	790	590
Donnelly	Erling	Wake Forest University	BA	3.16	520	770	780
Eminaga	**Seda**	Wisconsin, Univ Of--Madison	BS	3.98	500	770	660
Higa	**Leigh Ann**	Hawaii, Univ Of--Manoa	BA	3.93	720	750	730
Knight	Jefferson	North Carolina,U - Chapel Hill	BS	3.98	610	800	800
Li	Jia	Boston Univ		3.8	630	750	650
Lin	Michelle	Cornell University	BA	3.4	500	770	600
Massimine	**Kristen**	Bates College	BS	3.59	500	700	560
Patton	Brian	Missouri, Univ Of--Columbia	BA/BS	3.61	740	740	800
Urban	**Alexander**	Humboldt University	MD		520	640	630
Wittmack	Ellen	Iowa State University	BS	3.47	490	700	690
		Washington, University of	BA/BS	3.1	510	600	640
		SUNY At Stony Brook	BS	4	560	760	660
		Pennsylvania, University of	BA	3.9	550	800	800
		Carnegie Mellon University	BS	3.15	590	790	800
		Maryland, Univ Of--Baltimore	BS	3.47	420	750	650
		Brandeis University	BA	3.79	500	800	750
		Massachusetts, U Of--Amherst	BA	3.41	350	630	520
		Wisconsin, Univ Of--Madison	BS	3.12	450	620	730
		Springfield College (Mass)	BS	3.83	420	470	590
		Beijing University	BS		590	790	720
		National Taiwan University	BS		610	780	800
		Purdue Univ--West Lafayette	BS	3	410	590	470
		Beijing University	BS		650	790	770
		Ithaca College	BA	3.57	460	650	690
		Other Institution	BS		620	800	680
		Calcutta, University of	BS		700	660	780
		Puerto Rico, Univ Of--Mayague	BS	3.92	570	790	680
		Rutgers Univ--New Brunswick	BA	3.22	610	720	780
		Fudan University	BS		660	790	760
		Indian Inst of Tec, Kharagpur	BS		680	800	740
		Virginia, University of	BA	3.62	650	670	600
		Beijing Medical University	BS		570	790	640
		Johns Hopkins University	BA	2.55	540	740	750
		Other Institution	BS/MS		700	730	660
		Vassar College	BS	3.13	570	700	760
		Michigan, Univ Of--Ann Arbor	BS	3.32	400	720	730
		Kentucky, Univ Of--Lexington	BA	3.88	490	650	660
		Beijing University	BS		690	800	780
		Manhattan College	BS	3.82			
		Bowdoin College	BA	3.92	530	730	750
		Saskatchewan University	BS		530	560	490
		South Alabama, University of	BS	3.46	370	770	680
		Calif, Univ Of-Irvine	BS	3.19	460	760	770
		Nassau Community College	AA		500	610	550

		Univ of Science & Technology	BS		670	790	790
		Guelph, University of	BS		540	610	590
		Trinity College (Connecticut)	BS	3.3	530	710	690
		Florida, University of	BS	3.9	740	730	450
		Inst. Tecn. Autonomo de Mexico	MD		600	620	590
		SUNY At Stony Brook	BS	3.51	600	700	740
		Brigham Young Univ--Provo	BS	3.35	410	640	660
		William & Mary, College of	BS	3.3	610	640	770
		Delaware, University of	BS	3.76	440	720	730
		Beijing University	BS		440	710	720
		Nankai University	BS		690	790	690
		Beijing Medical University	BS		690	790	790
		Univ of Science & Technology	BS		490	780	730
		Yunnan Univeristy	BS		290	700	340
		North Carolina,U - Chapel Hill	BS	3.55	700	680	790
		Calif, Univ Of-Berkeley	BA	3.74	670	690	670
		William & Mary, College of	BS	3.84	610	740	690
		ALL		3.54	552	716	682
		ADMIT		3.61	539	735	685
		ADMIT/MATRIC		3.64	548	728	669

Table 1. Applicant pool. Class entering 2000-2001. Names are provided for students who were admitted and matriculated to the Pharmacological Sciences and Molecular Medicine Track of the BBS. Students named in bold were funded in year 1 by the **BCRTP**.

Class	Students	Rotations and Dissertation Advisors (underlined) **= BCRTP trainer
entered Fall 2000	Seda Eminaga Leigh-Ann Higa Kristen Massimine* Alexander Urban*	**Stern, **Rimm, **Bennett **Perkins, Bordey, Shlomchik, **Zhang Lolis, Lifton, **DiMaio Strittmatter, **Lizardi, Rakic,
entered Fall 1999	Jessica Hawes Soo-Jung Lee Julie Wu	(information provided in previous annual report)

Table 2. Students and rotations, 2000-2001.
*No longer funded by training program.
**BCRTP trainer

courses taken in year 1 or year 2	Hawes	Lee	Wu	Eminaga	Hi-ga	Massimine	Urban
Fall term.							
Advanced Biology Lab							•
Cell Biology 602a Molecular Cell Biology	•	•	•	•			
Genetics 625a Basic Concepts of Genetic Analysis	•	•				•	•
Pathology 680a seminar Topics in Molecular Medicine: Matrix Biology	•	•	•	•	•	•	•
Pharmacology I:Maintaining and restoring homeostasis			•		•		
Pathology 640a From Molecular Biology to Molecular Medicine							
Signal Transduction Seminar					•		
Apoptosis Seminar					•		
Physiological Systems I				•		•	
Medical Physiology							•
MBandB 752a Genomics/Bioinformatics							
Spring term.							
Pathology 650b Cellular and Molecular Biology of Cancer	•	•	•				
Biotechnology						•	
Pathology 660b Biology and Therapy of Breast Cancer		•	•	•	•		
Medical Physiology							•
Pathology 690b Mechanisms of Disease	•						
Pharmacology II: Interfering selectively	•	•	•	•	•	•	
Pharm 518b Current Topics in Cancer and Viral Therapy				•		•	
Pharm 502b seminar	•	•	•	•	•	•	•

Table 3. Coursework by trainees in years 1 and 2, 2000-2001.